

Reproduction, Development, Pheromone Secretion in *Heteromurus nitidus* Templeton, 1835 (Collembola, Entomobryidae).

BY

Susanne KROOL und Th. BAUER

Tierökologie I, Universität, Postfach 10 12 51, D-8580 Bayreuth, GFR

Synopsis: The life history of *Heteromurus nitidus* and its aggregation behaviour are described. The development, life span and reproductive behaviour of the species resemble those of other Entomobryidae. Aggregation pheromones are secreted exclusively by mature specimens.

Keywords: Collembola, *Heteromurus*, life history, aggregation, sexual pheromones.

INTRODUCTION

Springtails are popular objects for ecological and ecophysiological investigations of soil animals (cf. JOOSSE, 1983). A few investigations of the life history and behaviour of single species (for lit. see DUNGER, 1983) revealed interesting details, in particular with respect to reproduction in these insects. SCHALLER (1953) found that mating is indirect in springtails. WALDORF (1974) was able to show that spermatophores are more frequently deposited by males in the presence of mature females. MERTENS & BOURGOIGNIE (1977) and VERHOEF *et al.* (1977) proved the existence of weakly volatile and species specific pheromones which cause aggregation. Pheromones seem to be produced by both sexes (JOOSSE & KOELMAN, 1979), even during periods of starvation (VERHOEF, 1984). The site of production is apparently the digestive tract (VERHOEF, 1984).

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Evidently the main task of the aggregation pheromones of Collembola is to ensure the encounters of mature specimens of a species, since the spermatophores seem to lose their fertilization capacity some hours after deposition (cf. MAYER, 1957). Therefore we assume that only mature springtails produce pheromones causing aggregation.

We tested this hypothesis with *Heteromurus nitidus* (Templeton, 1835). This species belongs to the Entomobryidae and occurs in caves and the upper layer of soils of warm and temperate zones all over the world (PALISSA, 1964). As a hemiedaphic species it has a low resistance to desiccation (BAUER & CHRISTIAN, 1987). We have been maintaining this species in laboratory cultures for several years. This enabled us to observe isolated specimens from the egg to natural death, and even to experiment with populations of specimens of known age.

We shall first describe the life-history of *H. nitidus* and then describe experiments showing aggregation behaviour caused by specimens of different age.

I. — MATERIAL AND METHODS

The laboratory cultures of *Heteromurus nitidus* were kept at 20° C in containers with a bottom layer of moist plaster and fed with soya flakes. Freshly laid eggs were put singly in small glass vessels (Ø: 1 cm, height: 3 cm), which were filled with moist plaster to a height of 2 cm. The development was observed at 20° C under natural light conditions. The culture dishes were controlled daily under a binocular microscope, and body length and moultings were recorded. Sixty-six animals were observed in this way, 39 ♀♀ and 27 ♂♂.

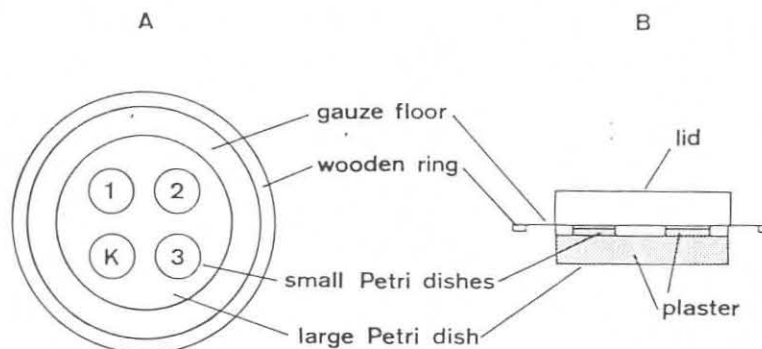


FIG. 1. — Experimental arrangement to test the aggregation of *Heteromurus nitidus*. A: from above, B: from the side; K: Petri dish containing springtails, 1-3: Petri dishes without springtails.

Aggregation experiments. Fig. 1 shows the experimental arrangement. A large Petri dish (Ø: 22 cm) was filled with moist plaster to a height of 1.4 cm below the rim. Four small Petri dishes (Ø: 5.5 cm, height 1.4 cm) were also filled with moist plaster to 3 mm

below the rim and put into the large Petri dish. A piece of gauze, stretched across a wooden ring was inserted as a kind of platform above the Petri dishes, and the large Petri dish was covered by a lid. When Springtails were placed on the gauze platform under the lid, they clustered in the areas above the 4 small Petri dishes because of the slightly higher humidity there due to the short distance between plaster and gauze.

The experiments were carried out in a dark room at 22° C. 150 to 200 springtails were placed on the gauze platform. Their distribution was recorded 15 times with intervals of 8 min from above by means of a camera with a flash attachment. To avoid contamination of the gauze floor it was turned and shaken after each recording. Care was taken that the animals were distributed homogeneously before the next recording. Between the single experiments the gauze was cleaned with detergent and water and then dried. In each experiment one of the small Petri dishes contained a number of springtails during or previous to the test period: a) 40 adults, enclosed for two days; b) 40 adults, enclosed for two days, animals, faeces and exuviae having been removed 1 h before the experiment by a blast of air; c) conditions as in b, but area cleaned one day before the experiment was started; d) 40 juveniles, 10 days old, enclosed for two days; e) as in d but juveniles 14 days old; f) as in d but juveniles 18 days old; g) as in d, but springtails 3-4 weeks old, just after reaching maturity.

In the control experiment the distribution was recorded with uncontaminated Petri dishes.

II. — RESULTS

A) Life-history.

The homogeneously round, white eggs of *Heteromurus nitidus* increase their diameter from 0,13 mm to 0,16 mm by water uptake within 48 hours after deposition. Then the chorion bursts around the equator. The now cylindrical egg (0,18 × 0,23 mm) is enveloped by the serosa, which bears characteristic protuberances. After 8 - 10 days (mean 9, n = 66) the young springtail tears up and strips off the serosa within ca 40 min. Its cuticula is till transparent, the scales which are typical in *H. nitidus* appear in later instars.

Freshly hatched juveniles measure 0,47 × 0,1 mm. Fig. 2 shows their stepwise increase from moulting to moulting until death. One to three days before each ecdysis the animals reduce their activity and stop feeding. Ecdysis itself takes only three to five min. One to three days after ecdysis they cast out the midgut epithelium. Sexual maturity is reached after the 5th - 6th ecdysis when the animals have obtained ca 65 % of their maximal body size. From then on growth is slower (cf. Fig. 2). During growth the body proportions change: the head increases by a factor of 3.1; the thorax grows 4.2 fold and the abdomen enlarges by a factor of 4.8.

The moulting intervals increase with age. The mean duration until the 6th ecdysis is 4.3 days, from the 7th to the 10th 6.5 days, from the 10th to the 16th 8.2 and from the 17th ecdysis on 9.5 days. Even with constant temperature and food supply the intervals vary considerably between individual animals. In the adult phase an interval may last between 3 days and 3 weeks. Often, but not always, short and long intervals alternate. The

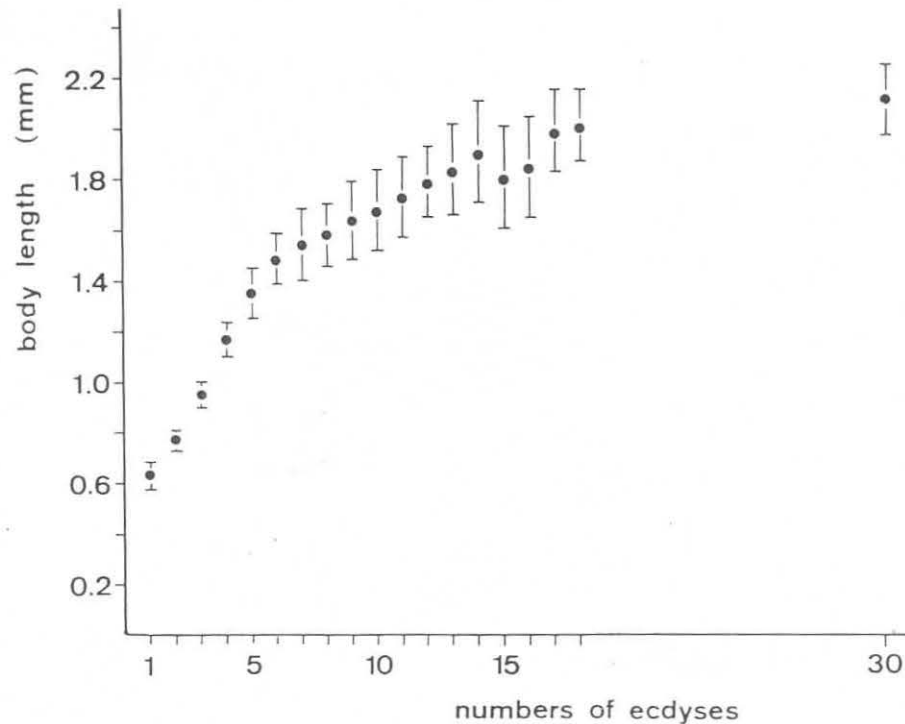


FIG. 2. — Body length of *Heteromurus nitidus* plotted against number of ecdyses; measure of dispersion standard deviation.

intervals of a juvenile are for example: 4,3,4,5,9,2,7 days, and for an adult: 14,3,20,6,11,7,11,7 days. Females reach a mean body length of 2,37 mm, males 1,85 mm. Most of the individually cultured animals died after 30 ecdyses and a life-span of approximately 9 months.

Sperm transfer occurs without contact of the sexes as in other Entomobryidae (MAYER, 1957). In dishes with isolated males, however, spermatophores were found only rarely. When males were brought together with mature females or enclosed in dishes that had been occupied by females before, it has been observed that the males deposited spermatophores in the way described by SCHALLER (1953). In one case a female was seen to move to the spermatophores and pick them up with slow movements of the abdomen. After four days it laid 21 eggs. Only twice eggs were found ($n = 14$) which had been laid by isolated (unfertilized) females. Though handled carefully these eggs did not show any signs of development and degenerated after a few days. This indicates that parthenogenesis apparently does not occur in *Heteromurus nitidus*.

B) Aggregation behaviour.

Fig. 3 shows the results of experiments with adult specimens. The highest percentage (64 %) of the experimental population aggregated above

adult specimens under the gauze floor (Fig. 3 B). The aggregation decreased to 46 % and 37 % respectively when the enclosure area was cleaned by a blast of air either one hour or one day before the experiment was started (Fig. 3 C and D). In the control experiment, in which none of the small Petri dishes had contained animals, no aggregation was observed (Fig. 3 A).

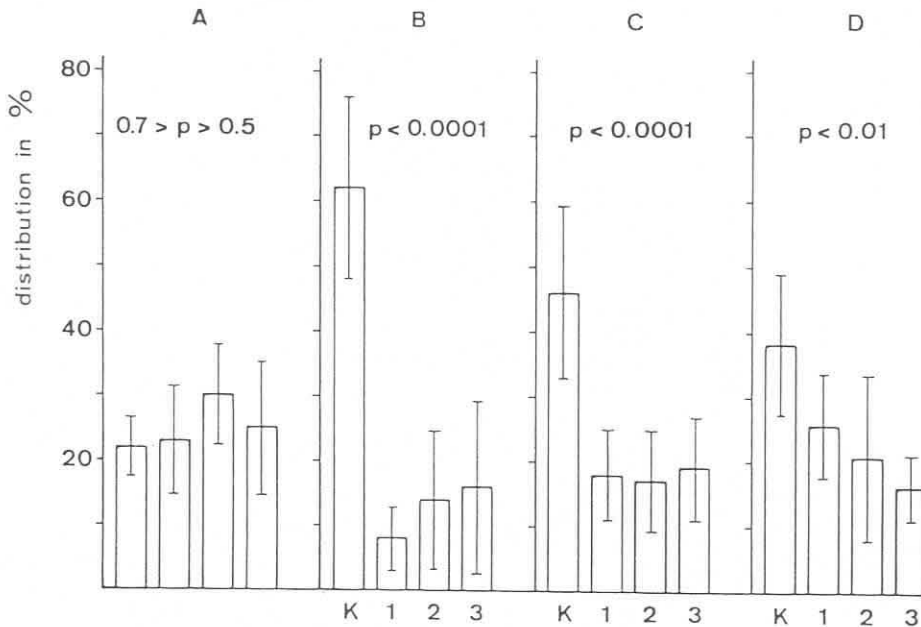


FIG. 3. — Mean distribution of the springtails on the gauze floor above the 4 Petri dishes; B: K with adult specimens, C: springtails from K removed one hour before the experiment, D: springtails from K removed one day before the experiment, A: control, Petri dishes uncontaminated; measure of dispersion: standard deviation; P: probability of accidental distribution (Chi — square test).

Aggregation was neither observed, when we enclosed juveniles of different ages in one of the Petri dishes under the gauze floor (Fig. 4 A - C). *Heteromurus nitidus* reaches maturity between the 5th and 6th ecdysis, at an age of 3-4 weeks. Specimens of this age caused aggregation (Fig. 4 D), indicating that aggregation pheromons are not secreted until maturity is reached.

III. — DISCUSSION

The development of *Heteromurus nitidus* is very similar to that of other Entomobryidae (cf. LINDENMANN, 1950; SCHALLER, 1953; MAYER, 1957; HALE, 1965; Joosse, 1970 a). Alternating moulting intervals of varying lengths of time as

described here were found in other species too (MAYER, 1957; JOOSSE, 1970 a). Exact data regarding the life span of single species were presented by MAYER (1957) who showed that the life time to Entomobryidae under laboratory conditions seems to be limited of about one year and the number of edyses to 30-40.

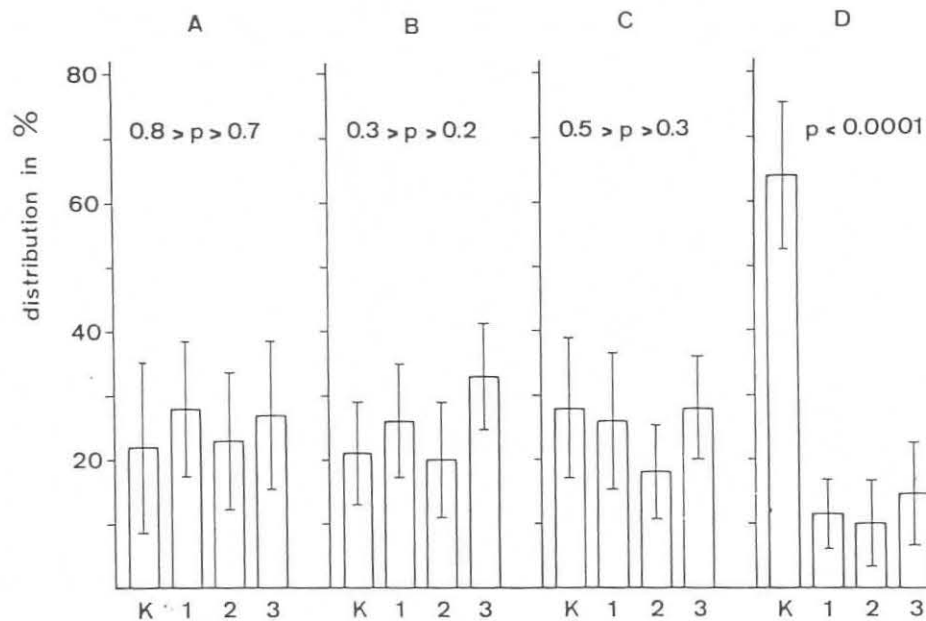


FIG. 4. — Mean distribution of the springtails above the Petri dishes when K contained young specimens. A: specimens in K 10 days old; B: 14 days old, C: 18 days old, D: 3-4 weeks old, just after reaching sexual maturity.

Under natural conditions two generations per year seem to occur normally although the strategies of overwintering are quite different (cf. LEINAAS 1981, ALLMEN & ZETTEL 1983, ZETTEL 1983, LEINAAS & BLEKEN 1983, LEINAAS & SOMME 1984, AITCHISON 1984).

MAYER (1957) distinguished four types of sexual behaviour in springtails. *H. nitidus*, like the other Entomobryidae, belongs to the second type: spermatophores are deposited without contact with a female, but preferably on sites where females are present. These sites are obviously marked by pheromones because spermatophores are also produced on sites where females were once present and then removed.

Aggregations in Collembola have been known for a long time (HALE 1965, JOOSSE, 1970). They may be a reaction to unfavourable environmental conditions. During dry weather populations of epedaphic and hemiedaphic springtails are forced to concentrate on moist places in the litter and during rain they swarm out again for feeding (JOOSSE, 1970 b; VERHOEF & NAGELKERKE,

1977; BOWDEN *et al.*, 1976; BAUER, 1979). But even under favourable conditions only 50-60 % of a population is engaged in feeding while the other part seems to be aggregated for moulting and reproduction (JOSSE, 1975; JOSSE & TESTERINK, 1977; BAUER, 1979).

According to VERHOEF (1984) aggregation pheromones are secreted by feeding as well as by inactive (moulting) specimens and even under conditions of starvation. However, the attractive force depends on pheromone concentration and thus on the density of individuals. This means that even the withdrawal sites of springtail populations are always marked by pheromones.

Our results prove that only mature specimens cause aggregation. The substances secreted are sexual pheromones which ensure the encounters of the sexes. It makes good sense that moulting, deposition of spermatophores and egg laying take place on marked sites where sufficient and continuous moisture is guaranteed. However, this is not necessarily the general pattern in Collembola. Atmobiote species for example, which are much more resistant to desiccation than the edaphic and hemiedaphic species may exhibit a quite different behaviour.

SUMMARY

Development, life cycle and behaviour of *Heteromurus nitidus* are described. Egg development takes nine days (at 20° C), sexual maturity is reached three to four weeks and five ecdyses, the life span amounts to approx. nine months (30 ecdyses). The aggregation behaviour was investigated with experimental populations (150-200 animals) on differently contaminated areas. Contamination with adult specimens caused aggregation, while contamination by young springtails until the fourth ecdyses did not.

We conclude, that the aggregation pheromones in *H. nitidus* are sexual pheromones which ensure the encounters of mature specimens.

RÉSUMÉ

Reproduction, développement et sécrétion de phéromone d'agrégation chez les *Heteromurus nitidus* Templeton 1835 (Collembola, Entomobryidae)

Le développement, le cycle de vie et le comportement a été étudié chez *Heteromurus nitidus*. Le développement embryonnaire dure 9 jours à 20° C. La maturité sexuelle est atteinte après la cinquième ecdysis, au bout de trois à quatre semaines. La durée de vie est de 9 mois, après 30 mues. Le comportement d'agrégation a été étudié sur des groupes de 150 à 200 animaux sur des surfaces différemment contaminées par les produits de l'activité d'individus précédemment introduits. Une contamination préalable par des animaux adultes aboutit à une aggrégation des individus introduits tandis qu'il n'a pu être mis en évidence aucune contamination par des jeunes animaux jusqu'à la quatrième ecdysis. Une contamination par des animaux ayant dépassé la 5^e mue (donc après maturation sexuelle) a produit une aggrégation.

Nous concluons que, chez *Heteromurus nitidus*, les phéromones d'agrégation, sont des phéromones sexuelles qui assurent la rencontre d'animaux ayant atteint la maturité reproductive.

ZUSAMMENFASSUNG

Fortpflanzung, Entwicklung und Pheromonsekretion bei *Heteromurus nitidus* Templeton 1835 (Collembola, Entomobryidae)

Entwicklung, Lebenszyklus und Verhalten von *H. nitidus* werden beschrieben. Die Eientwicklung dauert 9 Tage (bei 20° C), die Geschlechtsreife wird in 3-4 Wochen nach der 5. Häutung erreicht, die Lebensdauer beträgt ca 9 Monate bei 30 Häutungen. Das Aggregationsverhalten wurde mit Versuchstierpopulationen (n = 150 - 200) über unterschiedlich kontaminierten Flächen untersucht. Kontamination mit adulten Tieren führte zur Aggregation, mit Jungtieren bis zur 4. Häutung dagegen nicht. Kontamination mit Tieren nach der 5. Häutung (nach Erlangung der Geschlechtsreife) rief Aggregation hervor. Wir schließen, daß die Aggregationspheromone bei *H. nitidus* Sexualpheromone sind, die das Zusammentreffen reifer Tiere sichern.

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